Aggregates formation between CUG repeat RNA Sequences and MBNL1

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy caused by expansions of CTG repeats[1]. RNAs containing expanded CUG repeats interfere normal mRNAs through titration of the Muscleblind-like RNA binding protein 1 (MBNL1), resulting in the RNA-protein (RNP) aggregates[2]. Since its aggregation mechanism and chemical nature are still unclear, we have designed an in vitro system for the formation of RNP aggregates on DNA origami[3] by RNA molecules with over 1000 CUG repeats and MBNL1 proteins (Fig. 1). Analysis of the aggregates by means of the high-speed AFM measurement provides mechanistic aspects of RNP aggregates formation.

In this study, CUG repeat RNA with different repeat numbers (n = 10, 20, or 28) was transcribed in vitro, and their purity and length were investigated by PAGE. The hairpin-like structure of RNA was successfully synthesized and confirmed by fluorescence enhancement of CUG repeat RNA-binding molecules such as thioflavin T (ThT)[4] upon its binding to the RNA repeat unit. $(CUG)_n$ RNAs were then assembled on the rectangle DNA origami[3] and its assembly was examined by PAGE. MBNL1 and MBNL1**Δ**105, an MBNL1 mutant deleted the C-terminal 111 amino acid residues, were cloned from pGEX plasmids and expressed in *E. coli*, and purified by His-tag and GST-tag chromatography. The purified proteins were checked by SDS-PAGE before they were utilized for aggregation formation study. The aggregates of RNP were visualized by the high-speed AFM measurement. As a result, the decrease in height of RNP implied their successful binding on the DNA nanostructure. This preliminary study of CUG repeat RNA-MBNL1 protein aggregates show possibility to verify their mechanism. Particularly, what kind of RNP aggregates are formed depending on the number of CUG repeats will be clarified.

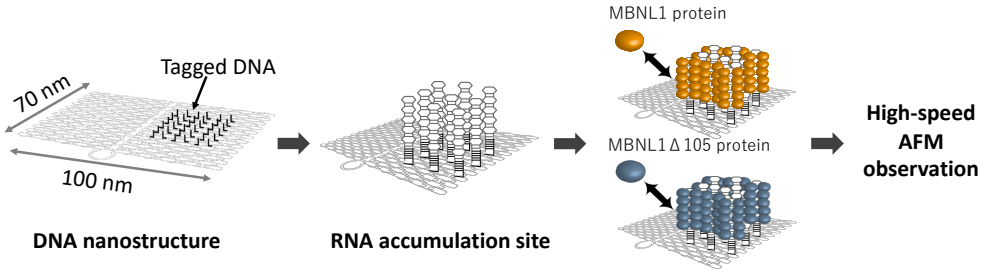

Fig. 1. Evaluating CUG repeat RNA-MBNL1 protein aggregates using DNA nanostructure.

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